Chronic infusion of amyloid-peptide and sustained attention altered 7 nicotinic receptor density in the rat brain
Chronic Infusion of Amyloid-β Peptide and Sustained Attention Altered α7 Nicotinic Receptor Density in the Rat Brain

Tania Araujo Viel,a,b,* Ariadiny Lima Caetano,c Marilia Silva Albuquerque,b Mariana Silva Araujo,d and Hudson Sousa Buckc

“School of Arts, Sciences and Humanities, Universidade de Sao Paulo, Av. Arlindo Bettio, 1000, Sao Paulo, SP, 03828-080, Brazil; bDepartment of Pharmacology, Institute of Biomedical Sciences, Universidade de Sao Paulo, Avenida Professor Lineu Prestes, 1524, 05508-900-Sao Paulo, Brazil; cDepartment of Physiological Sciences, Faculdade de Ciencias Medicas da Santa Casa de Sao Paulo, R. Dr. Cesario Motta Junior, 61, 11° andar, Sao Paulo, SP, 01221-020, Brazil; dDepartment of Biochemistry, Universidade Federal de Sao Paulo, R. Tres de maio, 100, 3° andar, Sao Paulo, SP, 04044-020, Brazil

Abstract: It is already known that progressive degeneration of cholinergic neurons in brain areas such as the hippocampus and the cortex leads to memory deficits, as observed in Alzheimer’s disease. This work verified the effects of the infusion of amyloid-β (Aβ) peptide associated to an attentional rehearsal on the density of α7 nicotinic cholinergic receptor (nAChR) in the brain of male Wistar rats. Animals received intracerebroventricular infusion of Aβ or vehicle (control - C) and their attention was stimulated weekly (Stimulated Aβ group: S-Aβ and Stimulated Control group: SC) or not (Non-Stimulated Aβ group: N-SAβ and Non-Stimulated Control group: N-SC), using an active avoidance apparatus. Conditioned avoidance responses (CAR) were registered. Chronic infusion of Aβ caused a 37% reduction in CAR for N-SAβ. In S-Aβ, this reduction was not observed. At the end, brains were extracted and autoradiography for α7 nAChR was conducted using [125I]-α-bungarotoxin. There was an increase in α7 density in hippocampus, cortex and amygdala of SAβ animals, together with the memory preservation. In recent findings from our lab using mice infused with Aβ and the α7 antagonist methyllycaconitine, and stimulated weekly in the same apparatus, it was observed that memory maintenance was abolished. So, the increase in α7 density in brain areas related to memory might be related to a participation of this receptor in the long-lasting change in synaptic plasticity, which is important to improve and maintain memory consolidation.

Keywords: Alpha7 nicotinic receptor, amygdala, amyloid-β, attention, hippocampus, memory, rehearsal.

INTRODUCTION

The involvement of the cholinergic system with cognitive processes has already been established. Activation of this system has been particularly involved in the amplification of long-term potentiation (LTP) induction [1-3] and, consequently, has been linked to learning and memory consolidation [4-6]. Particularly, α7 nicotinic cholinergic receptors (nAChR) have been recently implicated in hippocampal activity [7], memory reconsolidation [8] and sustained attention [9-11].

In some degenerative processes, like Alzheimer’s disease (AD), the cholinergic transmission is critically affected, which currently makes the anticholinesterase agents the pharmacological therapy of choice throughout the world [12-14]. Some studies have shown that activation of the cholinergic system in the hippocampus or in the amygdala may lead to cognitive adjustments related to emotional situations [15, 16]. This emotional-linked generated learning is associated to fear conditioning, which consists of a stressful process in which one learns to associate a stimulus with an eminent danger [17, 18], and keeps attention high to avoid danger. The acquisition and storage of information generated by fear conditioning can be related to LTP in the amygdala [18, 19]. Recently, it has been demonstrated that some of the nicotine-induced facilitation of LTP in this area is mediated by activation of NMDA glutamatergic receptors [20]. In addition to this mechanism, activation of nACh receptors located in GABAergic interneurons reduces the inhibition promoted by this system in the amygdala neurons, which in turn facilitates the activation of the glutamatergic system, and enhances LTP.

The density of α7 nACh receptors in the amygdala is greater than α4β2 density [21]. Also, functional nACh receptors in some amygdala nuclei (like the basolateral complex) are predominantly α7-containing receptors [22]. These receptors are believed to play an antiinflammatory and neuroprotective role in neurodegenerative diseases and in stressful situations [23-25]. Concerning these observations, the aim of this work was to determine the density of α7 nicotinic receptors in the hippocampus, cortex and amygdala of rats submit-
ted to the infusion of Aβ peptide and correlate this with sustained attention.

MATERIAL AND METHODS

Animals
Adult male Wistar rats (200 - 250 g body weight), provided from our own breeding colony, were kept in controlled room temperature (22-24 °C) and humidity (55-65%), with food and water *ad libitum* in a 12-hour-light/dark cycle. All the surgery and care procedures were strictly performed according to the guidelines for animal experimentation as stipulated in the Guide for the Care and Use of Laboratory Animals (National Institute of Health Publication number 86-23, Bethesda, MD) and The Ethics Committee on Experimental Research from Faculdade de Ciencias Medicas da Santa Casa de São Paulo, Brazil. All efforts were made to minimize the number of animals used and their level of suffering.

Behavioral tests
The active avoidance apparatus was used to evaluate memory evocation and also to stimulate the alert and sustained attention of the animals. Animals were selected according to their ability to learn and memorize a task [26]. The trials were performed in a two-way shuttle-box (Ugo Basile, Comerio, Italy) consisting of two compartments accessible to each other by a hole in the wall. Each animal was placed individually and then acclimated to the shuttle-box apparatus for 5 min before each session. The animal was then subjected to 50 trials of avoidance conditioning (acquisition test). Each trial consisted on 2 s conditioned stimulus (CS), i.e., a buzzer (70 dB, 760 Hz) and light, followed by 4 s unconditioned stimulus (UCS), such as a scramble shock of 0.5 mA delivered through the floor grid. Each trial was separated by fixed intertrial intervals (20 s). During the acquisition session, the number of conditioned avoidance responses (CAR), in which the animals moved to the other compartment of the shuttle-box before the beginning of the UCS, was recorded. All tests were carried out during the light phase (9:00 - 15:00 hr). In order to ensure that all animals submitted to the experiment had the same level of acquired memory, after one week (Test 1) animals were submitted again to the same protocol and those that reached a satisfactory learning performance - i.e., those achieving CAR rates between 30% and 70% [26] - were divided into four groups and submitted to the surgical procedures: 1) Non-stimulated control (N-SC, n = 5): animals infused with vehicle and left in cages with no stimulus; 2) Non-stimulated Aβ (N-SAβ, n = 6): animals infused with human 1-40 β-amylloid peptide + E-64 and left in cages with no stimulus; 3) Stimulated control (SC, n = 6): animals infused with vehicle (Hepes, 4 mM, pH 8.0) and submitted weekly to the same experimental protocol during the infusion period; 4) Stimulated Aβ (SAβ, n = 7): animals infused with human 1-40 β-amylloid peptide + E-64 and submitted weekly to the same experimental protocol during the Aβ infusion period. Weekly rehearsal was used to improve the sustained attention. The experimental design is showed in the timeline below:

Surgery
Selected animals were submitted to surgery for the implantation of mini-osmotic pumps (Alzet, model 2004, Cupertino, CA, USA) filled up with either the vehicle (4 mM HEPES, pH 8.0 - stimulated or non-stimulated control group) plus 0.8 μg E-64 or human (1-40) amyloid-β peptide (20 μg - stimulated and non-stimulated Aβ group) plus 0.8 μg E-64, following a method previously described [27]. E-64 is a cysteine protease inhibitor known for increasing neurodegeneration caused by Aβ infusion [26-29]. Briefly, after anesthesia with equitesin (4.25% chloral hydrate, 2.25% magnesium sulphate, 42.6%, propylene glycol 11.5% alcohol, 1% thiopental), a stainless steel cannula (Brain kit 1, Alzet, Cupertino, CA, USA) was implanted in the animal’s lateral ventricle using a stereotaxic instrument at coordinates: -0.8 mm anteroposterior, -1.4 mm mediolateral to bregma, and -3.5 mm dorsoventral to cranium [30]. The other extremity of the cannula was attached to a polyvinylchloride catheter (Medical grade, OD=1.14 mm, ID=0.69 mm) connected to the mini-osmotic pump that was subcutaneously implanted in the dorsum of the animal’s neck. The contents of the mini-osmotic pumps were delivered at a flow of 0.25 μL/hour, having a total volume of 200 μL, according to the manufacturer guidelines.

Five weeks later, the animals were anesthetized with CO₂ saturation, killed by decapitation and the brains were removed and immediately frozen in dimethylbutane at -50°C and stored at -80°C until use.
Autoradiography for α7 nAChR

The method used was based on a previously described procedure [31]. Serial sections of brains (20 μm) were cut on a cryostat chamber (-20°C to -22°C, Microm HM 505 N, Francheville, France), thaw-mounted on gelatin-coated slides, desiccated for 5 min at room temperature and kept at -80°C until use.

Sections were brought to room temperature (22°C) and air dried (5 -10 minutes). Incubations were conducted for 90 min at room temperature using 5 nM [125I]-α-bungarotoxin ([125I]- -BUTX, 143.2 Ci/mmOL). This concentration of radioligand was based on previous studies in the rat brain (data not shown) and corresponds to the Bmax value. Specific binding of the toxin for α7 nAChR, in this concentration, accounted for 80.2% of the total binding. Non-specific binding was assessed using 2 μM of the unlabelled toxin. The radioligand was diluted in 50 mM phosphate buffer containing 1 mM ethylenediiethyldiaacetec acid (EDTA) and 1 mM phenylmethyl-sulphonyl fluoride (PMSF), pH 7.4. At the end of the incubation period, slides were sequentially transferred through four rinses of 4 min each in the same buffer at 4 °C and rapidly dipped into cold distilled water to remove excess salts. Sections were air-dried and juxtaposed against Hyperfilm-MP (double-coated, 24 x 30 cm, Amersham Biosciences GE Healthcare, Uppsala, Sweden) for 14 days.

The method used was based on a previously described procedure [31]. Serial sections of brains (20 μm) were cut on a cryostat chamber (-20°C to -22°C, Microm HM 505 N, Francheville, France), thaw-mounted on gelatin-coated slides, desiccated for 5 min at room temperature and kept at -80°C until use.

Sections were brought to room temperature (22°C) and air dried (5 -10 minutes). Incubations were conducted for 90 min at room temperature using 5 nM [125I]-α-bungarotoxin ([125I]- -BUTX, 143.2 Ci/mmOL). This concentration of radioligand was based on previous studies in the rat brain (data not shown) and corresponds to the Bmax value. Specific binding of the toxin for α7 nAChR, in this concentration, accounted for 80.2% of the total binding. Non-specific binding was assessed using 2 μM of the unlabelled toxin. The radioligand was diluted in 50 mM phosphate buffer containing 1 mM ethylenediiethyldiaacetec acid (EDTA) and 1 mM phenylmethyl-sulphonyl fluoride (PMSF), pH 7.4. At the end of the incubation period, slides were sequentially transferred through four rinses of 4 min each in the same buffer at 4 °C and rapidly dipped into cold distilled water to remove excess salts. Sections were air-dried and juxtaposed against Hyperfilm-MP (double-coated, 24 x 30 cm, Amersham Biosciences GE Healthcare, Uppsala, Sweden) for 14 days.

The autoradiograms were quantified densitometrically using the MCID image analysis system (Interfoccus Europe, UK). For each specimen, α7 receptors binding sites were measured on 6 - 12 sections. The specific binding was determined by subtracting the non-specific binding from the total binding of adjacent sections.

Quantification of receptor binding sites

The autoradiograms were quantified densitometrically using the MCID image analysis system (Interfoccus Europe, UK). For each specimen, α7 receptors binding sites were measured on 6 - 12 sections. The specific binding was determined by subtracting the non-specific binding from the total binding of adjacent sections.

Drugs

Human (1-40) amyloid-β peptide was purchased from TOCRIS COOKSON INC, Ellisville, MO, USA, Na [125I]-α-BUTX from PERKIN-ELMER (Boston, MA, USA) and [125I]-α-bungarotoxin from SIGMA. All other drugs used were of analytical grade.

Statistical analysis

Results were expressed as means ± S.E.M. and analyzed using one-way analysis of variance (ANOVA) for multiple comparisons. The difference among treatments was analyzed using the post-test of Tukey-Kramer, as follows: Treatment 1: infusion of Aβ in non-stimulated animals (comparison between N-SAB and N-SC); Treatment 2: influence of attention training (comparison between SC and N-SC); Treatment 3: infusion of Aβ and attention training (comparison of SAB with N-SAB and SAB with SC).

For the behavioral analysis dissection, week by week, the two-way ANOVA followed by Bonferroni’s post-test was used.

RESULTS

Behavioral tests

Conditioned avoidance responses (CAR) of all animals registered one week after surgeries (Test 1, 67.2 ± 4.2%, n = 23) were similar (P = 0.1311). Using One-way ANOVA we observed significant differences among the CAR of the four groups analyzed (F3,22 = 11.61, P < 0.001). Treatment 1 significantly reduced the CAR for N-SAB (36.0 ± 5.8%, n=6, P<0.01) when compared to the CAR observed for N-SC (73.2 ± 7.4%, n=5) (Fig. 1). These findings were not due to alterations in spontaneous motor activity, since no changes were observed in locomotion performance of all animals (data not shown).

Treatment 2 did not alter the CAR observed for N-SC and SC groups. Treatment 3, however, maintained the CAR for SAB in values comparable to those obtained in Test 1 (68.6 ± 7.9%, n=7). These values were significantly higher (P<0.01) when compared to those obtained for N-SAB and not different from those obtained for SC (90.8 ± 1.8%, n=6) (Fig. 1).

Fig. (1). Conditioned avoidance responses (CAR) of rats registered in an active avoidance apparatus. Vehicle-infused animals are represented in blanked histograms, while Aβ-infused animals are represented in dark histograms bars. Histograms and vertical bars are means ± SEM. *: P < 0.05.

Dissection, week by week, of the behavioral analysis of stimulated groups

In order to better understand the animal’s behavior during the week rehearsal for sustained attention, we analyzed and compared the percentage of CAR in each of 10 trials of the 50 total trials observed each week.

In week 1 animals from Control or Aβ groups presented a statistically significant difference (F5,44 = 3.32, P < 0.05) along the fifth and fourth blocks of 10 trials, respectively, showing that they learned the trial. For the control group, in the following submissions to the equipment no difference in percentage of CAR was observed, showing that they kept the memory of the task. However, the Aβ group, in
the second week of observation, showed a lower percentage of CAR in the first 10 trials, which were significantly increased along the subsequent trials (P < 0.05). In the last three weeks, despite the lower performance of Aβ group, no statistical difference was verified between them and the control group (Fig. 2).

Quantification of α7 nAChR receptors

In the hippocampus of N-SC animals, specific binding sites for [125I]-α-BUTX were verified in pyramidal cells of CA1 area (Py: 0.3 ± 0.1 fmols/mg) and in the dentate gyrus (DG: 5.4 ± 0.6 fmols/mg). Other areas showed almost undetectable specific binding sites. Treatment 1 significantly increased the α7 density N-SAβ animals in Py (7.7 fold, P < 0.05) and in DG (2.0 fold, P < 0.01) (Fig. 3, “a” and “b” and Fig. 3, panels “a” to “d”). In the same way, Treatment 2 significantly increased [125I]-α-BUTX labeling in Py (14.3 fold, P < 0.001, Fig. 3a) and DG (3.3 fold, P < 0.001, Fig. 3b) of SC animals when compared to N-SC animals. After the stimulus, the SC group presented radioligand labeling (5.5 fmols/mg) in the CA3 area (Fig. 3c and Fig. 4, panels “g” to “k”). After Treatment 3, SAβ group showed a significant increase in α7 density (1.8 fold, P < 0.001) only in the dentate gyrus (Fig. 3b), when compared to N-SAβ. No difference in α7 density between SC and SAβ was observed.

In the amygdala of N-SC animals, labeling for [125I]-α-BUTX was verified in the lateral (0.9 ± 0.4 fmols/mg, Fig. 5a), basolateral (1.3 ± 0.7 fmols/mg, Fig. 5b), basomedial (4.3 ± 1.2 fmols/mg, Fig. 5c), medial (2.1 ± 1.0 fmols/mg, Fig. 5d) and cortical (8.2 ± 1.0 fmols/mg, Fig. 5e) nuclei. Infusion of Aβ peptide (Treatment 1) caused an increase in α7 density in all nuclei (including central nucleus - Fig. 5f), but a significant increase was observed only in the basolateral (5.0 fold, P < 0.01, Fig. 5b), medial (2.5 fold, P < 0.05, Fig. 5d) and cortical (2.1 fold, P < 0.01, Fig. 5e) nuclei (see also Fig. 5 to autoradiography pseudocolor pictures). Different from what was observed in the hippocampus, Treatment 2 caused no difference in the α7 nAChR density in SC animals, when compared to N-SC group. In Treatment 3, however, SAβ group presented significant increases in [125I]-α-BUTX labeling when compared to N-SAβ and SC groups in the lateral (2.0 fold, P < 0.001 and 2.6 fold, P < 0.001, respectively), basolateral (1.4 fold, P < 0.05 and 3.2 fold, P < 0.001, respectively) and basomedial (1.5 fold, P < 0.05 and 3.2 fold, P < 0.001, respectively) nuclei (Fig. 5a, b, c, respectively).

Concerning the central nucleus, no labeling for [125I]-α-BUTX was observed in N-SC animals (Fig. 5f and Fig. 6). Moreover, no difference in α7 receptor density was observed after any treatment applied.

Other areas directly related to memory that we also observed labeling for [125I]-α-BUTX in N-SC animals were the frontal (1.7 ± 0.7 fmols/mg) and temporal (1.3 ± 0.5 fmols/mg) cortices. After Treatment 1, no difference in α7 density in these areas was observed. However, Treatment 2 promoted significant increases in [125I]-BUTX binding of 2.0 fold (P < 0.001) in the frontal cortex (Fig. 7 and 8) and 3.0

![Fig. (2). Week dissection of conditioned avoidance responses (CAR) of Control and Aβ animals submitted to rehearsal in the active avoidance apparatus. For that, each 50 trials were divided in five groups of 10 trials. Histograms and vertical bars are means ± SEM. *: P < 0.05.](image-url)
Fig. (3). Specific binding of $[^{125}I] \cdot \alpha$-BUTX to $\alpha 7$ nicotinic cholinergic receptor in the pyramidal cells of CA1 area (a), dentate gyrus (b), CA3 (c) of hippocampus of the different animal groups. Data are presented as means ± SEM. *: P < 0.05; **: P < 0.01; ***: P < 0.001.

Fig. (4). Pseudocolor photomicrographs of autoradiograms representing anatomical distribution of total binding sites for $\alpha 7$ nACh receptor in the pyramidal cells of CA1 area and in the dentate gyrus of hippocampus of non-stimulated control (N-SC, a), non-stimulated Aβ (N-SAβ, b), stimulated control (SC, d) and stimulated Aβ (SAβ, e) animals and also in the CA3 area of hippocampus of N-SC (g), N-SAβ (h), SC (j) and SAβ (k) animals. High level binding of the antagonist to the $\alpha 7$ receptor is shown in yellow/red, according to the scale. Non-specific binding sites are represented in “f” and “l”. Structures identification is represented on panels “c” and “i” (adapted from Paxinos and Watson, 2007). The antero-posterior levels are approximately -2.92 mm to panels “a”, “b”, “d”, “e” and “f” and -4.44 mm to panels “g”, “h”, “j”, “k” and “l”, with reference to bregma (Paxinos & Watson, 2007). Abbreviations: cc, corpus callosum; cg, cingulum; GrDG, granular layer of the dentate gyrus; PoDG, polymorph layer of the dentate gyrus; MoDG, molecular layer of the dentate gyrus; Py, pyramidal cell layer of the hippocampus; fi, fimbria of the hippocampus; LV, lateral ventricle; VG, ventral geniculate nucleus; ic, internal capsule; sox, supraoptic decussation; Or, oriens layer of the hippocampus; Alv, alveus of the hippocampus; CA3, field CA3 of the hippocampus; SLu, stratum lucidum of the hippocampus; Rad, radiatum layer of the hippocampus; AH, amygdalohippocampal area; BLP, basolateral amygdaloid nucleus, posterior part; Co, cortical amygdaloid nuclei, posterior part; APir, amygdalopiriform transition area; DEn, dorsal endopiriform nucleus; LVI, lamina VI of cortex.
Fig. (5). Specific binding of $[^{125}I]^{-\alpha\text{-BUTX}}$ to $\alpha_7$ nicotinic cholinergic receptor in the lateral (a), basolateral (b), basomedial (c), medial (d), cortical (e) and central (f) nuclei of the amygdala of the different animal groups. Data are presented as means ± SEM.*: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

Fold ($P < 0.01$) in the lamina VI of temporal cortex (Fig. 7 and 4, in panels a, b, d and e) in SC animals. In the same way, Treatment 3 also promoted a significant increase in $\alpha_7$ density in those areas (1.9 fold, $P < 0.01$ and 1.6 fold, $P < 0.05$, respectively), when comparing to N-SAβ animals (1.8 ± 0.4 fmols/mg and 2.0 ± 0.4 fmols/mg, respectively).

Non-specific binding for $[^{125}I]-\alpha\text{-BUTX}$ in all groups was 0.6 ± 0.1 fmols/mg.

DISCUSSION

In the present work we observed that animals submitted to the chronic infusion of Aβ peptide presented a significant decrease in memory retrieval, whereas weekly submissions of similar animals to the active avoidance test promoted a significant improvement in memory retention, despite the neurodegeneration in hippocampus and cortex caused by infusion of Aβ (as we showed previously) [28]. This was
Pseudocolor photomicrographs of autoradiograms representing anatomical distribution of total binding sites for α7 nACh receptor in the amygdala of N-SC (a), N-SAβ (b), SC (d) and SAβ (e) animals. High level binding of the antagonist to the α7 receptor is shown in yellow/red, according to the scale. Non-specific binding sites are represented in “f”. Structures identification are represented on panel “c” (adapted from Paxinos and Watson, 2007). The antero-posterior levels are approximately -2.92 mm to panels “a”, “b”, “d” “e” and “f”, with reference to bregma (Paxinos & Watson, 2007). Abbreviations: H, hypothalamus; LH, lateral hypothalamus; opt, optic tract; GP, globus pallidum; CPu, caudate putamen (striatum); Ce, central amygdaloid nucleus; La, lateral amygdaloid nucleus; Me, medial amygdaloid nuclei; BM, basomedial amygdaloid nucleus; BL, basolateral amygdaloid nuclei; VEn, ventral endopiriform nucleus. For other abbreviations, refer to Figure 3.

Specific binding of [125I]-α-BUTX to α7 nicotinic cholinergic receptor in the frontal (a) and temporal (b) cortices of the different animal groups. Data are presented as means ± SEM.*: P < 0.05; **: P < 0.01; ***: P < 0.001.

RELATIONSHIP BETWEEN INCREASES IN α7 DENSITY AND MEMORY MAINTENANCE

It is well known that memory formation circuits are very complex, involving several brain areas. Situations that involve emotional requirement (like fear and stress, for instance) are more probable to be stored as long term memory, once associative learning is a suitable way to reinforce long term potentiation (LTP) and, in this way, to learning and memory [18, 32].

Two important brain areas related to this process are the amygdala and the hippocampus. The amygdala plays an important role in the connection of sensory information, particularly in relation to fear and anxiety, and promotes appropriate autonomic and behavioral reactions [33, 34]. It also has intensive interconnections with the hippocampal formation and cortical areas that are involved in memory processing [17, 18, 35]. In this study, animals were weekly submitted to an active avoidance apparatus. The fear-conditioning circuit implicated in this task involves the activation of neurons from the amygdala basolateral complex [34, 36] and, as a result, animals present reactions related to fear or defense [18, 37]. In a recent work, our research team showed that C57Bl/6 mice as well as mice lacking bradykinin B1 or B2 receptors showed an aging-related reduction response to this task [38].
Here, animals that were infused with vehicle (N-SC and SC) had the same performance in this behavioral test, independent of the number of times they were submitted to the test. However, SC animals showed a significant increase in $\alpha_7$ density in the hippocampus and the cortex, but not in the amygdala nuclei, when compared to the N-SC group. Apparently, SC animals did not demand the fear-conditioning circuits to avoid the shock, since their areas related to attention and memory consolidation (cortex and hippocampus) were intact [28]. There is evidence that LTP occurs in Py and DG [39, 40] and also that $\alpha_7$ receptors are involved with this process [1, 3, 5].

The involvement of $\alpha_7$ nicotinic receptors in sustained attention has already been described using $\alpha_7$ KO mice [9, 10] and rats treated with an $\alpha_7$ partial agonist (with 5-HT3 antagonist actions) [11]. Taken together, these observations suggest that control animals learned and stored the information they acquired during the intermittent submission to the active avoidance apparatus and that cholinergic neurons participated in that process.

It was already shown by our research team and others that infusion of Aβ peptide into rats’ lateral ventricle causes hippocampal and cortical neurodegeneration [28] and formation of amyloid plaques [41, 27] together with cognitive impairment [26] and LTP disruption [42, 43]. Using the same protocol, the cognitive impairment was also observed in mice, showing that this effect is not species specific [29]. Concerning human reports, there have been contradictory data related to $\alpha_7$ nAChR expression in brains of AD patients. While some works showed an evident reduction in the density of this receptor, others showed an increase in $\alpha_7$ nAChR- mRNA or no difference in $[125I]\cdot\alpha$-bungarotoxin binding (reviewed in [43] and [24]). The apparent contradiction of the $\alpha_7$ nAChR density in brain areas of AD patients related to memory can be related to the fact that hippocampal and cortical neurons that express $\alpha_7$ nAChR seem to be more vulnerable to neurodegeneration, once there is evidence that this receptor can bind to Aβ peptide, leading to Ca$^{2+}$ influx reduction, which contributes to cell death and cognitive impairment (for review, see [24]). However, there is suitable data that relate the increase in $\alpha_7$ nAChR to a non-defined compensatory mechanism of the brain [23, 24, 62].

We showed before that SAβ animals presented losses of cell bodies in hippocampal and cortical areas after infusion of Aβ [28]. Here, we observed an increase of $\alpha_7$ receptors only in the DG concerning the hippocampus. However, a striking increase in the lateral, basolateral and basomedial nuclei of the amygdala was verified (when compared to both N-SAβ and SC). As discussed above, these three nuclei formed the basolateral complex of the amygdala, which receives the influence of many neurotransmitter systems, such as noradrenergic, cholinergic, glutamatergic and gabaergic [48-51] and is clearly related to the emotional modulation of

Fig. (8). Pseudocolor photomicrographs of autoradiograms representing anatomical distribution of total binding sites for $\alpha_7$ nACh receptor in the frontal cortex of N-SC (a), N-SAβ (b), SC (d) and SAβ (e) animals. Non-specific binding sites are represented in “c”. Structures identification are represented on panel “f” (adapted from Paxinos and Watson, 2007). The antero-posterior levels are approximately 2,76 mm with reference to bregma (Paxinos & Watson, 2007). Abbreviations: DP, dorsal peduncular cortex; IL, infralimbic cortex; PrL, prelimbic cortex; Cg1, cingulate cortex, area 1; M1, primary motor cortex; M2, secondary motor cortex; Fr3, frontal cortex, area 3; S1J, primary somatosensory cortex, jaw region; Gl, granular insular cortex; DI, dysgranular insular cortex; AID, agranular insular cortex; LO, lateral orbital cortex; AccH, accumbens nucleus, core; AccSh, accumbens nucleus, shell; DEn, dorsal endopiriform nucleus; Pir, piriform cortex.
memory [34, 52]. The basolateral complex of the amygdala constitutes the entrance pathway for information that comes from sensorial stimulation (sound, light, shock, etc) [18, 53]. After that, it is transmitted to the central nucleus of the amygdala, which, in turn, projects it to areas of brain stem and hypothalamus that control defense behaviors, hormonal secretion and autonomic responses [37]. Interestingly, none of the animals showed labeling for α7 receptors in the central nucleus of the amygdala, suggesting that this receptor is involved with the input of the information, but not in the efferent response. The cholinergic plasticity in amygdala was previously observed with the chronic exposure to nicotine [20] and there is evidence that formation and store of information generated by fear conditioning in the amygdala also involve LTP [18, 19].

Finally, an increase in α7 nAChR was observed in the hippocampus, frontal and temporal cortices, mainly in the lamina VI of stimulated animals. Lamina VI was shown to be a network for corticothalamic and corticocortical communications [54] and has been associated to cognitive functions [55, 56].

Concerning the formation of LTP in hippocampus and in the amygdala, a probable pathway to explain the increase in α7 density in these areas and also in the cortex involves the stabilization of these newly formed synapses by neurotrophins, mainly the brain-derived neurotrophic factor (BDNF). Among its numerous synaptic actions, BDNF is involved in the regulation of α7 nicotinic receptors in the hippocampus and in parasympathetic neurons [57-59]. In hippocampal cultures, it was demonstrated that BDNF increases both surface and internal pools of α7nAChR and this increase is dependent on glutamatergic activity [57, 59]. Also, the increase in α7 could happen in both pre and postsynaptic regions. In fact, it was already shown that presynaptic α7 can regulate glutamate release in hippocampus and other regions, whereas postsynaptic α7 down-regulate inhibitory GABAergic interneurons activity necessary for long-term potentiation in the CA1 area of hippocampus [57, 59, 60, 61]. In addition to this, in recent findings from our group, C57Bl/6 mice were chronic infused with Aβ together with the α7 antagonist methyllycaconitine (MLA) in the lateral ventricle and the same behavioral protocol described in the present work (week stimulation in an active avoidance apparatus) was used. It was observed that the memory maintenance was abolished, reinforcing the involvement of the α7 receptors in the memory process (unpublished data). Quantification of α7, choline acetyltransferase and BDNF densities in brain areas of these animals are now being performed in our lab.

In conclusion, the observed increase in density of α7 nAChR in the hippocampus, cortex and amygdala of stimulated Aβ animals can be related to a long-lasting modification of the synapses which could contribute to the observed behavioral responses.

**INCREASES IN α7 DENSITY CAN ALSO BE RELATED TO A CENTRAL ANTI-INFLAMMATORY PATHWAY**

We also showed that chronic infusion with Aβ peptide, per se, caused an increase in α7 nAChR density in some hippocampal areas and some amygdala nuclei (as observed in N-SAB). This was also observed in other brain areas, either related to memory processes or not (data not shown). This increase in α7 density could be taken as an anti-inflammatory reaction to the presence of the Aβ peptide. In fact it was shown that the treatment of microglia cell cultures with acetylcholine or nicotine caused an antiinflammatory response via NFκB pathway [23, 44, 45]. This “nicotinic antiinflammatory pathway” was attributed to be dependent on the α7 nAChR, once the effect was attenuated with the antagonist α-bungarotoxin [23]. Similar observations were made in rats pre-treated with MLA and the anticholinesterasic agent donepezil and then, submitted to lipopolysaccharide (LPS) intracerebroventricular injection. MLA, but not dihydro-β-erythroidine (an α4β2 receptor antagonist) or scopolamine (a muscarinic receptor antagonist) reduced the anti-inflammatoryeffect of donepezil [46], showing, once again, the participation of α7 nAChR in the efferent way of the inflammatory reflex, which also involves the efferent vagus nerve and, obviously, the neurotransmitter acetylcholine [47]. This possible antiinflammatory reaction is being further investigated by our research team.

**CONCLUSION**

Sustained attention and Aβ infusion increased cholinergic neurotransmission in the cortex, the hippocampus and the amygdala via, at least, increases in α7 nAChR density. In agreement to other published articles, this might be involved with a modulation of the cholinergic system to induce LTP, leading to improvement of neuroprotection and memory maintenance. However, a compensatory mechanism promoted by the increase in α7 receptor density to avoid the neuroinflammatory process associated with the Aβ deposits was not discarded.

**CONFLICT OF INTEREST**

The author(s) confirm that this article content has no conflicts of interest.

**ACKNOWLEDGEMENTS**

The authors want to acknowledge the Fundação de Amparo à Pesquisa do Estado de Sao Paulo (FAPESP, Brazil) for the research funding. Ariadiny Caetano received a MSc fellowship from CAPES (Coordenação de Aperfeiçoamento Pessoal de Nível Superior, Brazil). Marília Albuquerque received an undergraduate fellowship from FAPESP. α-bungarotoxin was kindly provided by Dr. Rosely Godinho from Universidade Federal de Sao Paulo. The authors also would like to thank Dr. Wagner Montor, Department of Physiological Sciences, Faculdade de Ciências Médicas da Santa Casa de São Paulo for revising the English version.

**REFERENCES**


alpha7 nAChR Density and Memory

Current Alzheimer Research, 2012, Vol. 9, No. 10  1219


